REMARKS

The invention currently claimed relates to methods for the identification of a compound capable of modulating the expression or activity of a *daf-18* or PTEN gene. Such compounds are useful for ameliorating or delaying impaired glucose tolerance conditions or obesity, or increasing the longevity of a cell or organism.

Support for the Amendments

Claim 25 has been amended to correct the claim dependency and provide proper antecendent basis from pending claim 23. No new matter is added by this amendment.

Summary of the Office Action

Claims 1-5, 8-23, and 25-26 stand rejected under 35 U.S.C. § 112, first paragraph. This rejection is addressed below.

Claim Objection

Claim 25 is objected to because it depends from a canceled claim. In response to this rejection, Applicants have amended claim 25 to depend from pending claim 23.

Rejection under 35 U.S.C. § 112, First Paragraph

Claims 1-5, 8-23, and 25-26 stand rejected under 35 U.S.C. § 112, first paragraph, based on the assertion that Applicants' specification does not enable the presently claimed *daf-18* or PTEN screening methods, nor does it enable a transgenic nematode containing a transgene encoding a mammalian PTEN polypeptide.

The rejection of claims 1-5, 8-23, and 25-26 is based on the following three grounds: (1) that the specification fails to describe the regulatory regions of nematode *daf-18* and human PTEN gene promoters; (2) that the specification fails to make and use any transgenic nematode or mouse expressing a *daf-18* gene, a mutant *daf-18* gene, or a PTEN gene; and (3) that the specification fails to show that any *daf* gene is associated with the onset of impaired glucose tolerance, obesity, and longevity in other animals, especially mammals. The bases for the rejection are respectfully traversed.

The first basis for the § 112 rejection involves the assertion in the Office Action that the specification fails to describe the regulatory regions of nematode *daf-18* and human PTEN promoters. This basis for the rejection is respectfully traversed.

Applicants contend that standard methods for obtaining the regulatory sequences of a gene were known to one skilled in the art of molecular biology at the time the present application was filed. For example, at the time the application was filed, genomic library screening methodologies, PCR techniques, and genomic sequence database searches were commonly used by molecular biologists to obtain 5' flanking regions of a desired gene. Once the 5' flanking region is obtained, deletion mutants can readily be analyzed to

identify the important regulatory regions.

These standard techniques have, in fact, been used to clone the *daf-18* promoter. As attested to in the attached Declaration of Dr. Gary Ruvkun, the *daf-18* promoter was cloned using methods well known to a practitioner in the field of molecular biology. Using standard PCR techniques, a 6.9 kb DNA fragment was isolated from *C. elegans* N2 genomic DNA. This DNA fragment contains the *daf-18* gene as well as approximately 1.0 kb of 5' flanking sequence and approximately 1.1 kb of 3' flanking sequence. These flanking sequences contain regulatory elements that are sufficient to direct expression of *daf-18* and to rescue a *C. elegans daf-18* mutant.

In addition, as pointed out in the Reply to Examiner's Action filed October 26, 1999, the upstream regulatory region of human PTEN is readily available, for example, from the Entrez sequence database. In view of the above comments, Applicants respectfully request that this basis of the rejection be withdrawn.

The second basis for the § 112 rejection is based on the statement in the Office Action that the specification fails to make and use any transgenic nematode or mouse expressing a *daf-18* gene, a mutant *daf-18* gene, or the human PTEN gene. In particular, the Office states that the specification fails to show that a genomic DNA fragment encoding the *daf-18* or PTEN homolog can be inserted into a nematode or a mouse to produce the required phenotype of such transgenic animals. The Office Action further states that the specification fails to show that *daf-18* and PTEN have similar functions. This basis for the rejection is also respectfully traversed.

Applicants first point out that the pending claims do not recite the use of a transgenic mouse expressing a *daf-18* gene or human PTEN gene. Thus, this basis for the rejection is addressed in reference to nematodes, as recited in claim 5. Applicants contend that the pending claims that involve the use of a *daf-18* or PTEN transgenic nematode are enabled by the specification. For example, the specification, at page 38, provides a reference (Mello *et al.* EMBO J. 10:3959-3970, 1991) for generating transgenic *C. elegans*; such methods were standard in the field of nematode genetics at the time the present application was filed. In addition, the PTEN gene sequence was publicly available at the filing date of this application. Thus, one of skill in the art could readily generate a transgenic PTEN nematode.

With respect to the issue of functional similarity between *daf-18* and PTEN, the Examiner is again directed to the Ruvkun Declaration. As described in the Declaration, *C. elegans* expressing a PTEN polypeptide rescued the phenotype of *daf-18* mutants. This study was performed essentially as described in the specification. The human PTEN cDNA was placed under the control of approximately 1.0 kb of *daf-18* 5' flanking sequence and approximately 2.4 kb of *daf-18* 3' flanking sequence. Transgenic *C. elegans* (*daf-2*;*daf-18* double mutants) containing this minigene DNA were generated using standard methods known at the time the present application was filed. The F2 progeny of the transgenic *C. elegans* were then evaluated for restoration of the Daf phenotype (dauer formation at 25°C, but not at lower temperatures, for example, 20°C), which would indicate that rescue of the *daf-18* mutation was successful. The results of

these studies demonstrated that human PTEN fully rescued the dauer arrest phenotype of the *daf-2*;*daf-18* double mutants.

Accordingly, these *in vivo* results demonstrate that *daf-18* and PTEN function similarly, and therefore that either gene may be used in the presently claimed screening assays. The second basis for the rejection may be withdrawn.

The third and final basis of the § 112 rejection focuses on the statement in the Office Action that the specification fails to show that any *daf* gene is associated with the onset of impaired glucose tolerance, obesity, and longevity in animals other than *C. elegans*, especially mammals. The Office further states that methods of diagnosing an impaired glucose tolerance condition, obesity, or a propensity thereto by analyzing the level of PTEN expression or activity in a sample isolated from a patient is not allowed because PTEN's role in these conditions is not clear. This basis for the rejection is also respectfully traversed.

Applicants first submit that the evidence relating to the role of *daf* genes and the PTEN gene in insulin signaling is striking. The role of PTEN, in the regulation of insulin signaling in human cells has been established (Maehama et al., J. Biol. Chem. 273:13375-13378, May 29, 1998; submitted with the Reply to Examiner's Action filed October 26, 1999). This evidence directly indicates that, as a regulator of insulin signaling, PTEN is involved in impaired glucose tolerance conditions. In addition, the ability of PTEN to rescue a *C. elegans daf-18* mutation establishes that *daf-18* and PTEN are functionally similar. Therefore, there is no reason to believe that *daf-18* is not

similarly involved in impaired glucose tolerance conditions, or that such screening assays would not identify therapeutically useful candidate compounds.

Applicants further submit that the *daf* genes of the present invention, including *daf-18*, are involved in pathways for regulating glucose metabolism, the members of which are conserved from *C. elegans* to mammals. As described at pages 2-5 of the specification, two signaling pathways are required for the regulation of metabolism and dauer arrest in *C. elegans*: the DAF-2 insulin signaling pathway, and the DAF-7 TGF-β signaling pathway. *C. elegans* which are deficient in either the DAF-7 or the DAF-2 signaling pathway arrest at the dauer stage, indicating that both pathways are essential for regulating metabolism and dauer arrest.

As attested to in the Declaration of Dr. Gary Ruvkun, filed October 26, 1999, the TGF-β and insulin signaling pathways in humans and *C. elegans* share a large number of family members. For example, *C. elegans* gene products involved in the regulation of metabolism through the TGF-β signaling pathway include DAF-7, DAF-4, DAF-1, DAF-8, DAF-14, and DAF-3. Mammalian family members of this same pathway have also been identified. For example, DAF-7 is a particular subtype of the TGF-β superfamily; DAF-1 and DAF-4 are *C. elegans* members of the type I and II TGF-β receptor families, respectively; and DAF-3, DAF-8, and DAF-14 are *C. elegans* members of the Smad family of proteins.

The TGF-β signaling pathway acts in concert with the insulin signaling pathway to regulate metabolism and dauer arrest. Mammalian members of the insulin signaling pathway include insulin, the insulin receptor, PI-3 kinase, PDK1 kinase, AKT/PKB kinase, and the particular forkhead proteins FKHR, FKHRL1, and AFX. Again, *C. elegans* orthologs of many of these mammalian gene products have been identified. For example, DAF-2 is the *C. elegans* ortholog of the human insulin receptor superfamily; AGE-1 is the *C. elegans* ortholog of human PI-3 kinase; PDK-1 is the *C. elegans* ortholog of human PDK1; DAF-18 is the *C. elegans* ortholog of human PTEN; *C. elegans* AKT-1 and AKT-2 are orthologs of human AKT kinase; and DAF-16 is the *C. elegans* ortholog of human FKHR, FKHRL1, and AFX.

Moreover, Applicants point out that PTEN is not the only mammalian *daf* ortholog to function in *C. elegans*. Other human proteins of the insulin pathway can function in *C. elegans*, interacting with *C. elegans* proteins to regulate metabolism. For example, as attested to in the Declaration of Dr. Gary Ruvkun filed October 26, 1999, the human FKHRL1 gene, a member of the mammalian forkhead protein family that is a DAF-16 ortholog, can be expressed under the control of the *C. elegans daf-16* gene promoter in *daf-16* mutant *C. elegans*. When so expressed, the human FKHRL1 gene functionally complements the *daf-16* mutant.

Each of the above findings emphasizes the fact that human and *C. elegans* insulin signaling pathways are functionally similar, and that the *daf* genes which are members of this pathway are indeed involved in the regulation of impaired glucose tolerance conditions.

Similarly, with respect to insulin conditions involving obesity, Applicants again submit that a number of lines of evidence indicate that the *daf* and PTEN genes play roles in obesity conditions and that the presently claimed screening methods could indeed be exploited for the identification of anti-obesity compounds.

On this issue, Applicants first direct the Examiner's attention to the specification at page 62, lines 10-14. There, the specification describes a 14 year old diabetic insulin-resistant patient, who was morbidly obese. This patient carried the same insulin receptor mutation as a *C. elegans daf*-2 mutant (*e1391*) described in the present specification, demonstrating the association between *daf* genes and conditions involving obesity in humans.

In addition, Applicants again direct the Examiner's attention to the Declaration of Dr. Gary Ruvkun, filed October 26, 1999. There, Dr. Ruvkun points out that dauer arrest of various insulin signaling pathway mutants can be rescued by expression of a protein which complements the mutation, and that such a rescue also results in the loss of fat in the rescued animals. For example, *C. elegans daf-2* mutants are normally dauer-arrested and exhibit increased fat accumulation when compared to their wild-type counterparts. When the *daf-2* mutants are rescued from dauer arrest (for example, by mutation of the

daf-16 or *daf-18* gene) and therefore from an impaired glucose tolerance condition, they also exhibit lower fat accumulation levels. Similar results are observed in *C. elegans age-1* mutants. These mutants also exhibit increased fat accumulation as dauers, and, upon rescue from dauer arrest by expression of the wild-type AGE-1 protein, similarly exhibit lower fat accumulation levels.

With respect specifically to the role of PTEN in obesity, Applicants refer the Examiner to Figs. 38A-38F of Applicants' specification, where it is demonstrated that the *C. elegans* homolog of PTEN, *daf-18*, modulates the level of fat accumulation in *C. elegans*. The data presented in this figure shows that *daf-18* suppresses the fat accumulation phenotype of an *age-1* null mutant, providing direct evidence that *daf-18* is involved in the regulation of fat levels in nematodes. Applicants submit that, due to the structural and functional similarity between DAF-18 and PTEN (discussed above) and the known role of PTEN in the regulation of insulin signaling in human cells, PTEN, like *daf-18*, would be predicted to regulate fat accumulation and could successfully be used in the presently claimed screens for obesity-related compounds.

Moreover, Applicants submit that the relationship between impaired glucose tolerance conditions and fat accumulation in mammals is well established in the medical literature. The inability of a mammal to regulate metabolism results in a shift of its metabolism away from burning energy and toward metabolism of fat. The metabolism of fat, in turn, leads to other conditions in mammals, including obesity.

For all of the above reasons, Applicants submit that the relationship between the *daf* genes, including *daf-18*, as well as the PTEN gene, and impaired glucose tolerance conditions leading to obesity has been established, and this basis for the rejection may be withdrawn.

Finally, to address the contention in the Office action that the specification fails to show that *daf* genes regulate longevity in nematodes, Applicants refer the Examiner to pages 103-107 of the specification, where evidence of the role of *daf* genes in longevity is described. There, Applicants indicate that weak *daf-2* and *age-1* mutants that do not arrest at the dauer stage nevertheless live much longer than their wild-type counterparts. In addition, a *daf-18* mutation suppresses the long life span of the *daf-2* and *age-1* mutants. These results demonstrate that the insulin signaling pathway, of which *daf-2*, *age-1*, and *daf-18* are members, can modulate longevity. The specification further states that *age-1* null mutants are characterized by their longevity phenotype. These *age-1* null mutations are suppressed by *daf-18*(*e1375*). Again, these results clearly demonstrate that *daf* genes, including *daf-18,* play a role in the regulation of longevity in nematodes.

With respect to the role of PTEN in longevity, Applicants again refer the Examiner to the specification at pages 103-107, where the ability of *daf-18* to regulate longevity is detailed, as described above. Applicants submit that, due to the demonstrated functional similarity between DAF-18 and PTEN in the regulation of dauer arrest and longevity, one skilled in the art would predict that PTEN could be used in the presently claimed screening and diagnostic methods.

In sum, Applicants have demonstrated that the genes of the DAF pathways, and specifically *daf-18*, as well as the PTEN gene, function to regulate impaired glucose tolerance conditions, fat accumulation, and longevity, providing strong evidence that screens involving these genes and pathways would successfully identify compounds involved in such conditions. Applicants submit that this final basis for the § 112 rejection may be withdrawn.

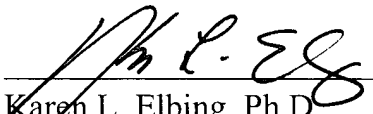
CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

Enclosed is a petition to extend the period for replying for three months, to and including July 10, 2000. If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 10 July 2000



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